TEMPERATURE-SENSITIVE MUTATIONS OF THE NOTCH LOCUS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Temperature-conditional mutations of the Notch locus were characterized in an attempt to understand the organization of a "complex locus" and the control of its function in development. Among 21 newly induced Notch alleles, about one-half are temperature-conditional for some effects, and three are temperature-sensitive for viability. One temperature-sensitive lethal, $l(1)N^{ts1}$, is functionally non-complementing for all known effects of Notch locus mutations and maps at a single site within the locus. Among the existing alleles involved in complex patterns of interallelic complementation, Ax^{59d5} is found to be temperature-sensitive, while fa^{g}, *spl*, and *l(1)N* are temperature-independent. Whereas temperature-sensitive alleles map predominantly to the right-most fifth of the locus, fa^{g}, *spl*, and *l(1)N* are known to map to the left of this region. Temperature-shift experiments demonstrate that fa^{g}, *spl*, and *l(1)N* cause defects at specific, non-overlapping times in development.—We conclude (1) that the Notch locus is a single *cistron* (responsible for a single functional molecule, presumably a polypeptide); (2) that the right-most fifth of the locus is, at least in part, the region involved in coding for the Notch product; (3) that the complexity of interallelic complementation is a developmental effect of mutations that cause defects at selected times and spaces, and that complementation occurs because the mutant defects are temporally and spatially non-overlapping; and (4) that mutants express selected defects due to critical temporal and spatial differences in the chemical conditions controlling the synthesis or function of the Notch product. The complexity of the locus appears to reside in controlling the expression (synthesis or function) of the Notch product in development.

GENETIC studies in *Drosophila melanogaster* have led to the discovery of genetically and developmentally "complex loci". Mutations at these loci express seemingly unrelated multicellular pleiotropic effects and exhibit complex patterns of complementation. Because the nature of the genetic and developmental complexity is unclear, "complex loci" have been variously interpreted as single genes (rudimentary, GREEN 1963; CARLSON 1971; Notch, WELSHONS 1965; maroon-like, CHOVNICK *et al.* 1969), as multiple genes with an operon-type organization (bithorax, LEWIS 1964), and as regulators of gene expression (BRITTEN and DAVIDSON 1969).

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Resolution of the complexity of the "complex locus" might provide new insights on the process of complementation in higher eukaryotes and might provide information on genetic organization in relation to regulation of gene action in development. This is specifically relevant to three currently important developmental problems: (1) substantiating the one gene-one chromomere model for chromosome organization that has been supported by systematic collections of lethal mutations within short chromosome segments (HOCKMAN 1971; JUDD, SHEN and KAUFMAN 1972); (2) understanding the multicellular pleiotropic expression of a gene; and (3) determining the role of the "excess" DNA of an average cytological unit or chromomere (RUDKIN 1965) and distinguishing between possible functions of this DNA in development (THOMAS 1971; LAIRD 1973).

The complex Notch locus in *Drosophila melanogaster* is especially suited for genetic analyses of developmental complexity. Mutations at this locus are known to affect multiple ectodermal derivatives, including nervous and sensory structures of larvae (POULSON 1940) and of adults (SHELLENBARGER 1971; FOSTER 1973). The locus mutates to both lethal and non-lethal forms, and all mutations lie within a single chromomere. Based primarily on the absence of complementation among lethal alleles and the distribution of these alleles throughout the mutable region, WELSHONS (1965) has concluded that Notch is a single *cistron* (responsible for a single functional product). However, something more complex is implied when the non-lethal alleles of this locus are considered. Although some non-lethal alleles can be considered as having lowered activity ("hypomorphs", WELSHONS 1965), this has not explained the expression of different sets of phenotypic defects and the complex patterns of complementation among Notch alleles. Recently, temperature sensitivity has been described for some mutations (FOSTER and SUZUKI 1970; SHELLENBARGER 1971; FOSTER 1973), and an extensive catalog of time- and tissue-specific requirements for the Notch gene and its product has been established by temperature-shift and genetic mosaic studies (SHELLENBARGER and MOHLER 1975).

In this report, the isolation and genetic characterization of temperature-conditional Notch mutations is described. We demonstrate that temperature-sensitive (ts) alleles map predominantly in the right-most fifth of the locus defined by all Notch mutations. The characterization of one ts-lethal mutation, $l(1)N^{ts1}$, which is functionally non-complementing for all known effects of the Notch locus, and which maps at a single site within the locus, strongly supports the conclusion that Notch is responsible for a single functional molecule and that at least the $l(1)N^{ts1}$ site may be involved in coding for a polypeptide.

We also demonstrate by temperature-shift experiments that all complexities of interallelic complementation at the Notch locus can be explained as the result of temperature sensitivity or of non-overlapping temporal and spatial defects. That mutations can cause non-overlapping defects in gene expression is due to developmental differences in the conditions for gene expression. This might occur at the level of product synthesis, in which case different mutations would lie in different control regions that function at different times or places. Or, the differences in conditions might be expressed at the level of product function, in

which case the different mutations would lie in the coding region for the Notch product, and each mutant product would lose activity in different times or places. Distinguishing between these possibilities will require isolation of the Notch product, which may be facilitated by use of ts alleles.

MATERIALS AND METHODS

Cultures of *Drosophila melanogaster* were grown on FRANKEL-BROSSEAU mix (1968) under defined conditions of temperature. Autosomes and their sources have not been controlled: thus, various strains and the new mutant strains are not isogenic. A detailed description of the mutants used in this study can be found in LINDSLEY and GRELL (1968).

Background genetics of Notch

The Notch locus (WELSHONS 1965; WRIGHT 1970) is located 3.0 map units from the distal end of the *X* chromosome. Cytological analyses of overlapping deletions localize Notch to salivary chromosome band 3C7 (SLIZYNSKA 1938) and/or an adjacent interband (WELSHONS 1974). The name "Notch" comes from the appearance of notches in wing tips in females homo- or heterozygous for certain mutations. Notch mutations may affect viability or may produce combinations of adult morphology defects, including notched wings, thickened wing veins with deltas at wing tip, extra or missing hairs and bristles, shortened tarsus, rough eye, and small eye. In general, non-lethal ("recessive visible") mutations have been classified as "eye" or

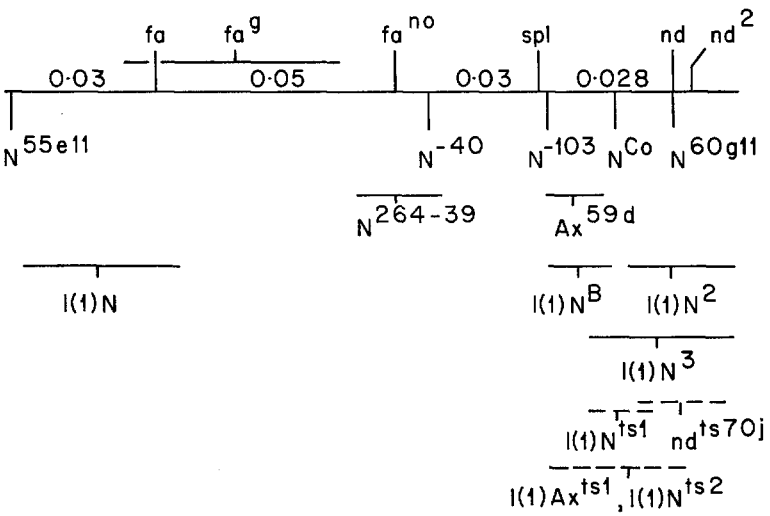

FIGURE 1.—Genetic map of the Notch locus (WELSHONS 1958a, b, 1965, 1971; WELSHONS and VON HALLE 1962; and this report). These mutations are recombinationally separable sites and all map to salivary chromosome band 3C7. Recessive visible mutations are placed above the line and separated by the percent recombination between them. Recessive lethals are below the line. Some lethals exhibit the dominant wing notches (*N*) or interrupted wing vein (*Ax*) phenotype in heterozygotes with an N^+ allele; others exhibit no defects in heterozygotes (symbolized *l(1)*, lethal, first chromosome). N^{-40} *is* N^{264-40}. N^{-103} *is* $N^{264-103}$. Horizontal lines indicate location of mutations not critically positioned. Mutations localized in this report are indicated by dashed horizontal lines. Mutations shown in this report to be temperature-sensitive (ts) are *fa*, fa^{no}, *nd*, nd^2, Ax^{59d}, nd^{ts70j}, $l(1)N^{ts1}$, $l(1)N^{ts2}$, and $l(1)Ax^{ts1}$. Temperature sensitivity of $N^{264-103}$ has been shown by FOSTER (1971). N^{60g11} is ts for wing notches and cold-sensitive for eye roughness in heterozygotes with N^+ (FOSTER and SUZUKI 1970).

"wing" mutations after the predominant phenotype expressed. WELSHONS (1958a, b, 1965, 1971) and WELSHONS and VON HALLE (1962) have localized many of these mutations in a linear sequence of recombinationally separable sites. A genetic map of Notch alleles is presented in Figure 1.

Complementation tests performed at 25° between Notch mutants demonstrate a complex functional relatedness of mutations. All heterozygotes[1] for two lethal mutations are non-viable. With the exception of the lethal mutation *l(1)N*, heterozygotes for all lethal mutations with all recessive visible mutations express the recessive visible phenotype. *l(1)N* complements recessive visible mutations (WELSHONS 1965). In addition, some heterozygotes for two recessive visible mutations are complementing. In general, eye/wing heterozygotes are normal, while wing-1/wing-2 heterozygotes are mutant for wing phenotypes. However, some eye-1/eye-2 combinations are mutant (e.g., *fa/fa*^{g}), while some are normal (e.g., *fa/spl* and *fa*^{g}*/spl*).

Abruptex (*Ax*) mutants express incomplete wing veins and missing bristles and hairs in heterozygotes (and in homozygotes if viable). Some alleles are homozygous lethal. *Ax* mutants are Notch locus alleles: they map within the mutational limits of the gene (WELSHONS 1971; FOSTER 1971), and they are non-complementing with other Notch lethals for viability (WELSHONS 1971). Like the *l(1)N* mutant, *Ax* mutants appear to be functionally unrelated to most recessive visible alleles in complementation tests at 25° (WELSHONS 1971).

Mutagenesis and recovery of new Notch mutations

Ethyl methane sulfonate (EMS) was made up fresh for each treatment and fed to Ore-R wild-type males by the method of LEWIS and BACHER (1968) (0.025 M in 1% sucrose). Procedure 1 (Figure 2a) was specifically designed to recover lethals (including ts lethals) in the white-Notch region of the *X* chromosome (SHELLENBARGER 1969). EMS-treated males were crossed with attached-*X* females carrying a *Y* chromosome ($w^{+}y^{+}Y$) containing the wild-type Notch region. Surviving males with putative lethal mutations (*X**) in the white-Notch region covered by the N^{+} duplication were crossed individually with N^{s}*/FM1* females, and N^{s}*/X** heterozygotes were tested for lethality at 29°. Strains that retested as lethal were saved as putative Notch mutants.

Procedure 2 (Figure 2b) was primarily designed to recover ts-lethals over the entire *X* chromosome. EMS-treated Ore-R males were crossed with attached-*X* females at 19°, and male progeny carrying the putative ts-lethal mutation were crossed individually with attached-*X* females at 29°, then subcultured at 19°. Strains producing males at 19° but not at 29° were identified as ts-lethal mutants. Male *X** chromosomes were then tested for lethality at 29° in N^{s}*/X** heterozygotes.

Fine structure recombination mapping

Three ts-lethal and one ts-notchoid allele were mapped relative to the fa^{no} and *spl* alleles carried in the w^{a} fa^{no} *spl rb* chromosome (kindly supplied by W. WELSHONS). ts-lethal/w^{a} fa^{no} *spl rb* females were crossed with *l(1)*N^{ts1} males for two to three days at 25°, then shifted to 29°. All males carrying a ts-lethal allele (½ of all male embryos), all females carrying two ts-lethal alleles (½ of all female embryos), and about 90% of females heterozygous for fa^{no} and a ts lethal are killed at 29°. The few surviving females were discarded. Surviving males include w^{a} fa^{no} *spl rb* non-recombinants which are viable at 29° and all recombinants which do not carry a ts-lethal (½ of all recombinant males); these males were scored for eye color and Notch locus phenotypes. Because fa^{no} expression varies, absence of its phenotype was inconclusive in some cases. These potential recombinants and all other recombinants between Notch alleles were kept and crossed with attached-*X* females for retesting at 19° and 29°. Map order was determined by the number and types of recombinants. Recombination distances were assigned using the ratio of recombinants in two small adjacent segments: fa^{no}*–spl* and *spl–ts lethal*. fa^{no} is 0.03 map units from *spl* (WELSHONS 1958a).

l(1)N^{ts1} was also mapped relative to N^{60g11} following a similar procedure. In this case, the only survivors were those receiving a recombinant chromosome containing neither *l(1)*N^{ts1} nor N^{60g11}. The data are used to assign a map order for these alleles.

[1] The term "heterozygote" in this report always refers to the *trans* form: *mutant-1* +/+ *mutant-2*.

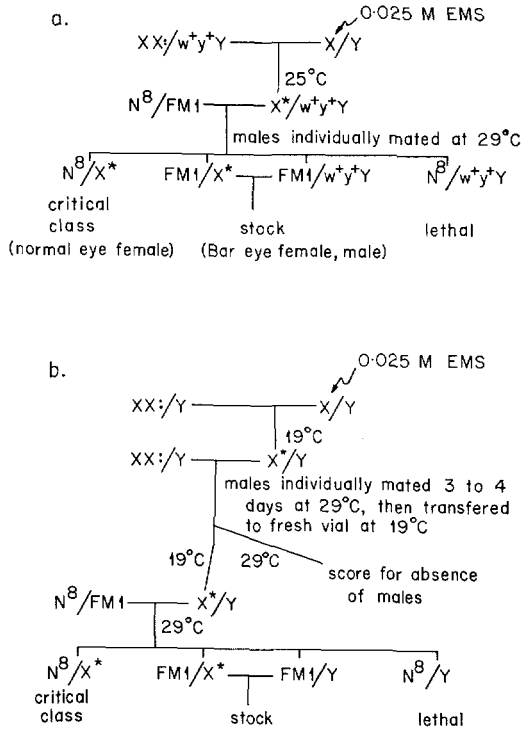

FIGURE 2.—Mating schemes for recovery of lethals and ts lethals at the Notch locus. (a) Procedure 1: recovery of lethals (including ts lethals) in the white–Notch region of the *X* chromosome. (b) Procedure 2: recovery of ts lethals in the white–Notch region from among ts lethals over the entire *X* chromosome. *XX:*=attached-*X* chromosomes. *=putative lethal. w^+y^+Y=*Y* chromosome with *X*-fragment carrying w^+, y^+, and N^+. N^8=*X* chromosome deleted for white–Notch and beyond. *FM1*=*X* chromosome balancer containing the dominant Bar eye mutation.

RESULTS

EMS induces temperature-conditional Notch mutations in high frequency

Twenty-one new Notch locus mutations were recovered and are classified by temperature sensitivity in Table 1. Of 4,340 EMS-treated chromosomes that survived in w^+y^+Y males (Procedure 1, Figure 2a), 19 are lethal as heterozygotes with the N^8 deletion at 29° (SHELLENBARGER 1969). Unexpectedly, all 19 are Notch locus mutations (the only other mutations recovered were six white non-lethal alleles and one roughest semi-lethal). Of 4,565 EMS-treated chromosomes that survived at low temperature (Procedure 2, Figure 2b), 143 contained ts-lethal mutations, two of which were lethal in heterozygotes with the N^8 deletion at 29°. Both of these, $l(1)Ax^{ts1}$ and nd^{ts70j}, are Notch locus mutations.

Assignment of the mutations to the Notch locus is based on survival in males carrying w^+y^+Y and on the following criteria in females: (1) lethality in heterozygotes with the N^8 deletion at 29°; (2) lethality in heterozygotes with the Notch point mutation $N^{264\text{-}39}$ or other representative Notch tester at 29°; and (3)

in some cases, expression of characteristic wing notches in heterozygotes with an N^+ allele (carried in the *FM1* chromosome) or in homozygotes.

Approximately one-half of the new Notch mutations are temperature-sensitive for certain effects under conditions of continuous culture at 29° and 18° (Table 1). The effects studied include lethality in females in one dose (heterozygous to the N^s deletion, which is considered a null allele) and in two doses (homozygous), and expression of wing notches in heterozygotes. Six classes are distinguished as follows:

Notch (N): homozygous lethal; wing notches in heterozygotes.

TABLE 1

Classification of 21 new Notch mutations by temperature effects

Mutant and class (N^x)	Survival* of females				% Notch wing in heterozygotes	
	N^x/N^s		N^x/N^x			
	29°	18°	29°	18°	29°	18°
Notch						
N^{68j1}	0	0	0	0	77	99
N^{68j2}	0	0	0	0	98	98
N^{68l}	0	0	0	n.t.†	93	100
N^{69d1}	0	0	n.t.	0	99	99
N^{69d2}	0	0	0	0	95	99
N^{69d3}	0	0	0	0	95	99
N^{69d4}	0	0	0	0	99	99
N^{69f1}	0	0	0	0	88	99
ts Notch						
ts N^{69c}	0	0	0	0	3	26
ts N^{69d5}	0	0	0	0	68	17
ts N^{69e1}	0	0	0	0	97	9
ts N^{69e2}	0	0	0	0	73	1
lethal						
$l(1)N^{69e3}$	0	0	0	0	1	0
ts lethal						
$l(1)N^{ts1}$	0	7	0	96	0	0
$l(1)N^{ts2}$	0	5	0	97	0	0
$l(1)Ax^{ts1}$	0	n.t.	0	44	Ax‡	Ax
ts facet-notchoid						
fa^{no69d6}	0	0	38	80	0	0
ts notchoid						
nd^{ts69js}	1	100	64	100	0	0
nd^{ts69d7}	19	85	65	100	0	0
nd^{ts69f2}	0	70	4	33	0	0
nd^{ts70j}	0	n.t.	30	95	20	0

* Measured as % relative to number of sibling females $N^x/FM1$. The number of $N^x/FM1$ females ranged from 19 to 300.

† n.t.=not tested.

‡ Abruptex phenotype, 100% penetrance at both temperatures.

ts Notch (*ts N*): homozygous lethal; temperature-sensitive wing notches in heterozygotes.

lethal (*l(1)N*): homozygous lethal; normal wings in heterozygotes.

ts lethal ($l(1)N^{ts}$): temperature-sensitive lethality in homozygotes; normal wings in heterozygotes, except in the case of $l(1)Ax^{ts1}$.

ts facet-notchoid (fa^{no}): temperature-sensitive semi-lethality in homozygotes; normal wings in heterozygotes.

ts notchoid (nd^{ts}): temperature-sensitive lethality in one dose and in homozygotes; normal wings in heterozygotes, except nd^{ts70j}.

Many of the ts alleles also cause the expression of morphology defects in surviving homozygotes. Although $l(1)N^{ts1}$ and $l(1)N^{ts2}$ homozygotes are normal at 18°, $l(1)Ax^{ts1}$ homozygotes at 18° express the *Ax* phenotype more extremely than do heterozygotes. fa^{no69d6} homozygotes at 29° express weak notches and deltas at wing tips, but at 18° they express strong notches and thick wing veins; for this mutant, the lethal effects are heat-sensitive and the wing defects are cold-sensitive. nd^{ts70j} homozygotes at 29° express wing notches, eye roughness, and semi-lethality phenotypes; at 18° they are completely normal. nd^{ts68j3}, nd^{ts69d1}, and nd^{ts69f2} homozygotes express weak notches, mild deltas, and variable extra bristles at both 18° and 29°.

Many recessive visible alleles are ts

The effect of temperature on the expression of adult morphology defects by the recessive visible alleles (*fa*, fa^{g}, *spl*, *nd*, nd^{2}, and fa^{no}) has been studied in homozygous females at 18°, 25° and 29°. These phenotypes are described in Table 2. Descriptions in LINDSLEY and GRELL (1968) correspond roughly to phenotypes at 25°. Only two alleles, fa^{g} and *spl*, are found to be temperature-independent. *fa* and *nd* are heat-sensitive for their respective phenotypes. fa^{no} expression is variable; generally, notching of wings is less extreme at 29° than at 18° or 25°; thick wing veins and deltas are expressed at all temperatures. nd^{2} is heat-sensitive in degree of notching of wings and for *spl*-like eye roughness and size, but it is cold-sensitive for the Abruptex phenotype!

ts lethals map to the right-most fifth of the Notch locus

$l(1)N^{ts1}$: Recombination analysis (Table 3) shows that $l(1)N^{ts1}$ is a single-site mutation located between *spl* and N^{60g11}, and that lethality and adult morphology defects (revealed by temperature-shift experiments, SHELLENBARGER 1971; SHELLENBARGER and MOHLER, 1975) map at the same site. Firstly, recovery of w^{a}–*N* and *N*–*rb* recombinants in roughly expected frequencies is good evidence that $l(1)N^{ts1}$ is a single mutation at the Notch locus. An inversion, translocation, large deletion, or second lethal mutation not at the Notch locus would be expected to reduce drastically the frequency of one or the other or both eye color recombinants. However, as seen in Table 3A, w^{a}–*N* recombinants (fa^{no} *spl rb*) occur with a frequency of 1.5% and *N-rb* recombinants (w^{a} fa^{no} *spl*) occur with a frequency of 5.4%; these values are close to the standard map distances of 1.5 for w^{a}–*N* and 4.5 for *N*–*rb*.

TABLE 2

Temperature-dependent expression of adult morphology defects in recessive visible homozygotes

Recessive visible allele	Temperature (°C)	Phenotypes in homozygous females
facet (*fa*)	29	eyes rough and splotchy; low penetrance and expressivity of apical nicks
	25	weak* eye roughness
	18	weaker eye roughness, almost normal
facet-glossy (*fa^{g}*)	29	eyes rough, glossy surface
	25	same
	18	same
split (*spl*)	29	eyes small and rough, pebbly surface; some bristles doubled, others (ocellar, supraalars, presutural) missing; some extra hairs
	25	same
	18	same
notchoid (*nd*)	29	eyes weakly small and rough; extreme apical and marginal notches; thick wing veins
	25	eyes weakly rough; notches; thick wing veins
	18	eyes normal; wings have weak nicks
notchoid-2 (*nd^{2}*)	29	eyes rough and small (*spl*-like); extreme apical and marginal notches; thick wing veins; shortened tarsus; semi-lethal as late pupa
	25	eyes *spl*-like; apical notches; thick wing veins
	18	eyes weak *spl*-like; weak nicks; incomplete wing veins; missing bristles; semi-lethal as late pupa
facet-notchoid (*fa^{no}*)	29	weak thick veins with deltas at vein tips
	25	thick wing veins with deltas; apical notches
	18	thick wing veins with deltas; apical notches

* weak=defects not maximally expressed.

Secondly, the number and types of recombinants unambiguously determine the map order *fa^{no} spl l(1)N^{ts1}* (Table 3A) and *l(1)N^{ts1} N^{60g11}* (Table 3B). Recombinants between Notch and the flanking eye color markers (*w^{a} fa^{no} spl* and *fa^{no} spl rb* recombinants) would be observed for any order of *fa^{no}*, *spl*, and *l(1)N^{ts1}*. However, the surviving recombinants between *l(1)N^{ts1}* and *fa^{no}* or *spl* differ critically for the three possible orders: (1) for the order *l(1)N^{ts1} fa^{no} spl*, single recombinants would have been *w^{a}*; (2) for the order *fa^{no} l(1)N^{ts1} spl*, single recombinants would have been *spl rb*; and (3) for the order *fa^{no} spl l(1)N^{ts1}*, single recombinants would have been *spl rb* and *rb*. As seen in Table 3A, there is a large number of *spl rb* and *rb* recombinants and no *w^{a}* recombinants, consistent only with the third order, *fa^{no} spl l(1)N^{ts1}*. Concerning the map order for *l(1)N^{ts1}* and *N^{60g11}*, the recovery of two *w^{a}* recombinants (Table 3B) indicates that *l(1)N^{ts1}* lethality is to the left of *N^{60g11}*.

TABLE 3

Fine structure recombination mapping of 1(1)N^{ts1} *relative to* fa^{no}, spl, *and* N^{60g11}: *number and types of recombinant chromosomes recovered in males*

A. $\frac{+\ +\ +\ l(1)N^{ts1}\ +}{w^a\ fa^{no}\ spl\ +\ rb}$ ♀♀ × $\frac{+\ +\ +\ l(1)N^{ts1}\ +}{Y}$ ♂♂

Male progeny genotype					Number recovered*	
w^a	fa^{no}	*spl*	+	+	2591	
+	fa^{no}	*spl*	+	*rb*	717	
w^a	+	*spl*	+	*rb*	1	(1)
+	+	*spl*	+	*rb*	29	(25)
+	+	+	+	*rb*	16	(16)
+	+	+	+	+	2	(2)

Total tested (male) chromosomes=47,610

Map position†: $l(1)N^{ts1}$ is 0.018 map units to the right of *spl*

B. $\frac{+\ l(1)N^{ts1}\ +\ +}{w^a\ +\ N^{60g11}\ rb}$ ♀♀ × $\frac{+\ l(1)N^{ts1}\ +\ +}{Y}$ ♂♂

Male progeny genotype				Number recovered	
w^a	+	+	+	2	(1)

Total tested vials‡=1,041

Map position: $l(1)N^{ts1}$ is to the left of N^{60g11}

* The number in parentheses is the number of recombinants retested at 18° and at 29°. All fa^{no} ambiguities were resolved toward fewer recombinants.

† Map distance= (18 *spl*–$l(1)N^{ts1}$ recombinants/30 fa^{no}–*spl* recombinants) × 0.03, where 0.03 is the standard map distance fa^{no}–*spl*.

‡ Non-recombinant chromosomes are lethal; # vials × # $l(1)N^{ts1}/Y$ males surviving at 18° (10 to 15) gives an estimate of the chromosomes tested.

Usually map distances are computed as percent of recombination events. Here, the distance fa^{no}–*spl* is 0.06 (30/47,610), which is twice the distance (0.03) found by WELSHONS (1958a). This difference is unexplained. However, the presence of both fa^{no} and *spl* markers provides another way of measuring distance—comparison of the relative number of recombination events within the two adjacent regions fa^{no}–*spl* and *spl*–$l(1)N^{ts1}$ using 0.03 as the standard map distance for fa^{no}–*spl*. By this second method, $l(1)N^{ts1}$ is 0.018 map units to the right of *spl*.

Finally, the inability to separate lethality from adult morphology defects (SHELLENBARGER 1971; SHELLENBARGER and MOHLER, 1975) in these viable recombinants localizes the morphology defects to the same site as lethality. If adult morphology defects mapped at a second site to the left of *spl*, then the *rb* recombinants (Table 3A) would be expected to show the defects at 29°. However, *rb* recombinants are phenotypically completely N^+; therefore, morphology defects map to the right of *spl*. If a second site mapped to the right of N^{60g11}, both w^a recombinants (Table 3B) would be expected to show adult morphology defects at 29°. However, they are completely N^+; therefore, morphology defects map to the left of N^{60g11}.

Therefore, $l(1)N^{ts1}$ is a single-site mutation exhibiting all the pleiotropy of the Notch locus.

$l(1)N^{ts2}$ and $l(1)Ax^{ts1}$: These mutations have been mapped relative to fa^{no} and *spl*; both mutations follow an identical pattern as that observed for $l(1)N^{ts1}$. Based on 37,000 tested chromosomes, relative recombination frequencies place $l(1)N^{ts2}$ 0.011 map units to the right of *spl*. $l(1)Ax^{ts1}$ maps 0.013 units to the right of *spl* based on 24,000 tested chromosomes. These distances are consistent with a map location to the left of N^{60g11}; however, this conclusion has not been tested. (Other *Ax* mutants have been mapped between *spl* and N^{60g11} by WELSHONS 1971).

nd^{ts70j}: This allele, originally isolated with a ts lethal located to the right of *rb*, has been mapped relative to fa^{no} and *spl*. Based on 13,000 tested chromosomes, nd^{ts70j} maps at the extreme right boundary of the Notch locus, 0.037 map units to the right of *spl*. However, it has not been tested for position relative to the right-most boundary mutant, nd^{2}.

In summary, 11 ts alleles are known to map at sites within the Notch locus (see Figure 1). Of these, all six lethal alleles and the three non-lethal alleles with the most extreme temperature effects are located in the right-most fifth of the locus. Excluding the fa^{no} allele, which has a highly variable expression at all temperatures, the *fa* allele is the only ts allele in the remaining four-fifths of the locus. Therefore, that fifth of the mutable region at the right end is, at the least, more highly mutable to both ts and non-ts alleles, and it may be functionally distinct.

ts lethal alleles are temperature-sensitive for adult morphology defects

Heterozygotes containing any ts-lethal allele ($l(1)N^{ts1}$, $l(1)N^{ts2}$, or $l(1)Ax^{ts1}$) and any recessive visible allele (*fa*, fa^{a}, *spl*, *nd*, nd^{2}, or fa^{no}) are non-complementing at 29° for the recessive visible defects. The phenotypes of these heterozygotes are described (Table 4) relative to the phenotypes of the homozygotes for recessive visible alleles (Table 2). In some heterozygotes, defects are expressed weakly; in others additional defects are expressed which are not observed in the recessive visible homozygote. The additional defects expressed in heterozygotes with N^{s} indicate that the full extent of the defects of a recessive visible allele is often greater than indicated in homozygotes. Because the defects in heterozygotes containing a recessive visible allele and a ts-lethal allele are in each case less extreme than in heterozygotes containing the null allele N^{s} (Table 4), it is concluded that the ts lethals are not complete null alleles at 29°.

At 18° ts-lethal alleles complement all recessive visible alleles. Although the phenotypes of $l(1)N^{ts1}/fa^{no}$ and $l(1)N^{ts2}/fa^{no}$ heterozygotes are not completely normal, these same phenotypes (low penetrance and expressivity of wing nicks and thick wing veins with deltas) are also weakly expressed in $fa^{no}/+$ heterozygotes. Similarly, the phenotypes of $l(1)Ax^{ts1}$ heterozygotes with recessive visible alleles are not normal, but are typical of $l(1)Ax^{ts1}/+$ heterozygotes. All other combinations are normal at 18°.

TABLE 4

Phenotypes of trans *heterozygotes containing ts-lethal alleles (or* N^s*) and recessive visible alleles at 29°*

Recessive visible allele	Phenotypes* in heterozygotes with			
	$l(1)N^{ts1}$	$l(1)N^{ts2}$	$l(1)Ax^{ts1}$	N^s
fa	fa	weak	weak	fa, N, exB
fa^g	fa^g	weak	weak	fa^g, N
spl	weak†	spl	spl	spl, nicks
nd	nd	nd	weak‡	nd, exB
nd^2	nd^2	weak	weak	lethal§
fa^{no}	nd^2	fa^{no}, N	N	lethal¶

* The symbols fa, fa^g, spl, nd, nd^2, and fa^{no} refer to phenotypes indistinguishable from homozygotes at 29° (defined in Table 2). weak=expression in heterozygotes is not as extreme as that in the corresponding homozygote. N=strong apical notches. nicks=weak apical notches. exB=extra bristles or hairs.

† Some legs have shortened tarsal segments.

‡ Expression of Abruptex phenotypes is extreme.

§ Animals die as late pupae which have the nd^2 phenotype.

¶ Animals die before the late pupal stage.

Therefore, the ts-lethal alleles are temperature-sensitive for expression of adult morphology defects characteristic of recessive visible Notch alleles.

ts lethal alleles share at least one developmental defect with Notch embryonic lethals

Heterozygotes containing any ts-lethal allele and any representative recessive lethal mutation (N^x: Notch, ts Notch, Abruptex, lethal, or ts lethal) do not survive to the emerged adult stage when cultured continuously at 29°. By this assay of survival, measured relative to survival of sibling females in crosses of the type *ts lethal/FM1* ♀ ♀ × N^x/w^+y^+Y ♂ ♂, there is no evidence of "complementation". All alleles must, therefore, share at least one developmental defect causing lethality.

There is strong evidence that the common defect of ts-lethal alleles and other lethal Notch alleles is in embryonic development. Firstly, $l(1)N^{ts1}$ and $l(1)N^{ts2}$ have effective lethal phases (time of arrest of development, HADORN 1961) within the embryonic to early larval stages. Only ⅓ of $l(1)N^{ts1}$ embryos hatch at 29°. Similarly, the lethal phase of all Notch lethal alleles except $l(1)N^B$ is in embryonic development; $l(1)N^B$ animals die in early larval stages (POULSON 1940, 1967, 1968). Secondly, $N^{sog11}/l(1)N^{ts1}$ heterozygotes at 29° die as embryos, indicating that these two alleles share a defect in embryonic development. Thirdly, temperature-shift experiments, in which $l(1)N^{ts1}$, $l(1)N^{ts2}$, or $l(1)Ax^{ts1}$ homozygotes are cultured at 29° only during embryonic development, indicate that these alleles cause defects during embryonic development: the "temperature-sensitive period" (SUZUKI 1970) for lethal effects of these alleles is in embryonic development (SHELLENBARGER and MOHLER 1975 and unpublished observations).

There is some evidence of partial complementation when the crosses with ts-lethal alleles are assayed for survival to the late pupal stage (Table 5). Specifically, the late pupae observed in matings of $l(1)N^{ts1}$ or $l(1)Ax^{ts1}$ with $l(1)N^{2}$, $l(1)N^{3}$, or $l(1)N^{69e3}$ clearly "complement" (survive beyond) the effective lethal phase: homozygotes for these alleles do not reach the late pupal stage. (Because some homozygotes for the $l(1)N^{ts2}$ allele reach the late pupal stage, combinations of alleles involving $l(1)N^{ts2}$ cannot be clearly interpreted.)

For those cases of partial complementation with respect to the embryonic defect, death at the late pupal stage indicates at least a second lethal phase, and presumably a second period of development which requires Notch function for survival: late pupae do not complement in this period. This second develop-

TABLE 5

Viability of heterozygotes containing ts-lethal and Notch-lethal alleles at 29°: assay of late pupae

Notch allele (N^x)	Survival* homozygous (N^x/N^x)	Survival* of heterozygotes $l(1)N^{ts1}/N^x$ %	Phenotype	$l(1)Ax^{ts1}/N^x$ %	Phenotype
Notch					
$w^a\ N^{55e11}\ rb$	n.t.†	0		0	
$w^{ch}\ N^{264\text{-}39}$	0	0		1	normal‡
$w^a\ N^{264\text{-}40}\ rb$	0	0		0	
ts Notch					
$w^a\ N^{60g11}\ rb$	0	0		0	
$ts\ N^{69d5}$	0	1	small eye; stubby leg	0	
$ts\ N^{69e2}$	0	1	headless	0	
Abruptex					
$w^a\ Ax^{59d5}$	0	0		10	Ax
lethal					
$l(1)N^{2}$	0	7	headless; fused stubby legs	50	small eye, head; Ax; fused leg
$l(1)N^{3}$	0	14	small eye, head; rough eye; fused stubby leg	29	small eye, head; Ax; stubby leg
$l(1)N^{B}$	n.t.	0		n.t.	
$l(1)N^{69e3}$	0	5	small rough eye; stubby leg	27	small eye, some rough; Ax
ts lethal					
$l(1)N^{ts1}$	0	—		2	stubby leg; Ax
$l(1)N^{ts2}$	8§	0		40	Ax
$l(1)Ax^{ts1}$	0	1	normal‡	—	

* Measured as % relative to $N^x/FM1$ emerged adult sibs. Ax=Abruptex.
† n.t.=not tested.
‡ Presumed to carry w^+y^+Y ($N+$) by nondisjunction.
§ Phenotype of late pupae: head-eyeless; rough eye; stubby leg; irregular chaetae.

mental requirement is clearly established by temperature-shift experiments for $l(1)N^{ts1}$ (SHELLENBARGER and MOHLER 1975). It is interesting that heterozygotes for $l(1)N^{ts1}$ and the one lethal mutation which permits survival beyond embryonic development, $l(1)N^{B}$ (POULSON 1968), do not survive to the late pupal stage. Either $l(1)N^{B}$ homozygotes die after hatching due to an embryonic defect shared by $l(1)N^{ts1}$, or the two alleles are defective at a common time after hatching of the larva.

At 18° ts lethals do not provide completely normal function. Heterozygotes containing the most severe class of lethals, the "Notch" alleles, do not survive to either the emerged adult or the late pupal stage. This is clearly in contrast to the survival of the "Notch" alleles in heterozygotes containing an N^{+} allele. Heterozygotes involving "ts Notch" alleles show reduced viability and survivors have notched wings and small rough eyes. Heterozygotes involving "lethal" alleles survive and are normal or have notched wings and small rough eyes.

Heterozygotes containing $l(1)N^{ts1}$ and $l(1)Ax^{ts1}$ appear to exhibit negative complementation at 18°; that is, the heterozygote does not survive as well as either homozygote. In this case, survival of $l(1)N^{ts1}$ and $l(1)Ax^{ts1}$ homozygotes is 96% and 44%, respectively, while survival of heterozygotes is only 26%. This is clearly allele-specific because $l(1)N^{ts2}$ homozygotes and heterozygotes with $l(1)Ax^{ts1}$ have better than 90% survival. This phenomenon has been reported previously for heterozygotes involving two Ax alleles (FOSTER 1971) and has been called "contracomplementation" (PORTIN 1974).

Temperature sensitivity accounts for interallelic complementation involving some recessive visible alleles

Results of complementation tests at 29° (Table 6) demonstrate three unam-

TABLE 6

Interallelic complementation of recessive visible alleles at 29°

Recessive visible allele	Phenotypes* in heterozygotes with							
	l(1)N	*Ax^{59d5}*†	*fa*	*fa^{g}*	*spl*	*fa^{no}*	*nd*	*nd^{2}*
fa	n.t.	fa	fa					
fa^{g}	+	fa^{g}	fa	fa^{g}				
spl	+	spl	+	+	spl			
fa^{no}	+	N	+	+	+	fa^{no}		
nd	n.t.	nd	+	+	+	fa^{no}, nicks	nd	
nd^{2}	n.t.	weak nd^{2}	nicks	+	spl	N, fa^{no}	nd	nd^{2}

* The symbols fa, fa^{g}, spl, fa^{no}, and nd^{2} refer to phenotypes indistinguishable from homozygotes at 29° (see Table 2). + = normal (completely complementing). N = strong apical notches. nicks = weak apical notches. weak = expression in heterozygotes is not as extreme as that in the corresponding homozygote. n.t. = no test.

† Phenotypes of all heterozygote combinations with Ax^{59d5} also include interrupted wing veins and missing bristles.

biguous types of complementation involving recessive visible alleles: (1) eye/wing, (2) eye–1/eye–2 (*fa^{g}/spl* and *fa/spl*), and (3) lethal/recessive visible. The results of tests at 25° and 18° reflect the temperature sensitivity of the alleles in any pair mating; thus, heterozygotes containing *fa* and *fa^{g}* are mutant at 29° but express a normal phenotype at 18° due to temperature-sensitive expression of the *fa* allele.

The complementation reported for heterozygotes at 25° containing a visible allele and *Ax^{59d5}* (WELSHONS 1971) is not repeated at 29°, indicating that *Ax^{59d5}* is temperature-sensitive for adult morphology defects. This is not surprising because some *Ax^{59d5}* homozygotes survive at 18°, indicating temperature sensitivity for lethal effects. Indeed, at 25° highly variable results have been obtained for the expression of both *fa^{g}* and *spl* in heterozygotes with *Ax^{59d5}* (a result also now observed by WELSHONS, personal communication). Intermediate and variable expression of alleles that are temperature-sensitive or that express new phenotypes in heterozygotes with *N^{s}* (Table 4) suggest that mutations may be interacting functionally at a level fluctuating near the visual perception of the investigator, and that such mutations may be hypomorphs (WELSHONS 1965).

Spatial and temporal non-overlap of defects accounts for the remaining complexity of interallelic complementation

Eye and wing mutations cause morphology defects that are spatially non-overlapping. Clearly, complementation of an eye with a wing mutation can occur if the defects in function of the mutant alleles are also spatially non-overlapping (GREEN 1964). That the requirements for normal Notch function in eye and wing development are independent and localized in each tissue, respectively, has been shown by autonomy of function in genetic mosaics (SHELLENBARGER and MOHLER 1975). Therefore, eye and wing mutations can complement because their defects are spatially non-overlapping.

Temperature-shift experiments (Table 7) identify the times in development when *fa*, *fa^{g}*, *spl*, and *l(1)N* do not meet the requirements for a normal Notch gene. Complementation among pairs of eye mutations can occur because mutants are defective at non-overlapping times in development. Complementation involving *l(1)N* can occur because this mutation is not defective after hatching of the larva.

Whereas *fa* homozygotes display the mutant phenotype at 29°, they are nearly normal at 18°. When 18° cultures are shifted up to 29° at pupariation, *fa* is expressed; however, when 29° cultures are shifted down to 18° at pupariation, *fa* is not expressed (Table 7). Therefore, *fa* is defective in pupal development but permits normal development in the larval stage.

Because *fa^{g}* homozygotes and *spl* homozygotes are mutant at both 18° and 29°, these alleles have been challenged by temperature shifts as heterozygotes with *l(1)^{ts1}*: high temperature inactivates the *l(1)N^{ts1}* product so that development depends on the recessive visible allele. *fa^{g}/l(1)N^{ts1}* heterozygotes are *fa^{g}* at 29° but normal at 18°. When 18° cultures are shifted up to 29° at pupariation, the *fa^{g}* phenotype is expressed; however, when 29° cultures are shifted down to 18°

TABLE 7

Phenotypes of fa, fa^g, spl, *and* l(1)N *in temperature-shift experiments*

Temperature protocol*	Phenotypes† in homozygotes			Phenotypes† in heterozygotes with *l(1)N^{ts1}*		
	fa	*fa^g*	*spl*	*fa^g*	*spl*	*l(1)N*
18° continuous	+‡	fa^g	spl	+	+	+
Shift-up						
18°—lh /—29°	n.t.	n.t.	n.t.	n.t.	n.t.	+
18°—wpp /—29°	fa	fa^g	spl	fa^g	+	+
Shift-down						
29°—wpp \—18°	+‡	fa^g	spl	+	spl	n.t.
29° continuous	fa	fa^g	spl	fa^g	spl	*l*

* Shifts were done at larval hatch (lh) or at pupariation (wpp, formation of the white prepupa).

† Symbols of phenotypes as in Table 7. *l*=lethal.

‡ Expression not normal, but clearly less extreme than continuous 29°.

at pupariation. the *fa^g* phenotype is not expressed (Table 7). Therefore, as was the case for *fa*, *fa^g* is defective in pupal development but permits normal development in the larval stage.

Similarly, *spl/l(1)N^{ts1}* heterozygotes are *spl* at 29° but are normal at 18°. However, the results of the shifts are reversed: the *spl* phenotype is expressed in shift-down experiments but not in shift-up experiments. Therefore, *spl* is defective in larval development but permits normal development in the pupal stage.

Clearly, normal function in *fa/spl* and *fa^g/spl* heterozygotes is to be expected because the eye mutations of each pair are defective at non-overlapping times in development.

Because *l(1)N/l(1)N^{ts1}* heterozygotes at 29° die as embryos, only shift-up experiments have been used to challenge the *l(1)N* allele at times after hatching of the larva (Table 7). These treated animals survive and have normal phenotype. Thus, the *l(1)N* allele is not defective at times in development after hatching of the larva. Complementation of *l(1)N* with recessive visible alleles therefore occurs because the mutations have non-overlapping temporal defects.

DISCUSSION

Notch is responsible for a single functional molecule

The *trans* complementation test assays whether two recessive mutations are defective in a common function or in different independent functions. The

identification of one function or many is ambiguous, however, when two mutations complement each other but are non-complementing with a third mutation, as is the case for some mutations at the Notch locus. Two complementing mutations (e.g., fa^9 and *spl*) would identify independent functions in cases when the third mutation (any lethal allele except *l(1)N*) is defective in both functions, as would be the case for a deletion, multisite, polar, or other *cis*-acting mutation. Because the $l(1)N^{ts1}$ mutation maps at a single site to one side of the fa^9 and *spl* alleles, non-complementation with both fa^9 and *spl* is clearly not by a deletion or multisite effect. In addition, the location of lethal sites throughout the Notch locus is not consistent with unidirectional polar or *cis*-acting effects for all lethal sites. Therefore, the Notch locus appears to be responsible for a single function or possibly multiple functions which are not independent (as multiple catalytic sites of a polypeptide). In either case, it appears that the functional product of the Notch locus is a single molecular species.

Interallelic complementation for non-overlapping developmental defects

A major argument against the conclusion that the Notch locus codes for a single functional molecule has been complementation between recessive visible alleles or *l(1)N*. Our studies with ts mutations demonstrate that these cases of interallelic complementation can be accounted for in terms of non-overlapping temporal or spatial defects of a single functional molecule in development (Table 7). The non-overlapping defects explanation is consistent with the extensive time and tissue specificity of the independent developmental requirements for expression of the Notch gene and/or activity of its product established by temperature shift and genetic mosaic studies (SHELLENBARGER and MOHLER 1975). The beauty of this explanation is that it points out and provides a simple answer to a critical basic question concerning mutants of genes with multicellular pleiotropic effects: "how can a single mutant allele be defective in one aspect of development but provide normal function in another aspect of development?" The simple answer is that chemical conditions for synthesis or function of the Notch gene product differ in the different times and places where Notch function is required, in a way critical to completion of Notch function.

Thus, for a given aspect of development with its specific set of conditions, one mutation blocks Notch function while a different (complementing) mutation provides normal function. For a given mutation, conditions in one aspect of development block Notch function while conditions in another aspect of required, in a way critical to completion of Notch function.

Although this simple explanation is sufficient to explain complementation and how mutations can cause selected multicellular defects at Notch and perhaps other complex loci, it does not exclude the possibility of other partial explanations. For example, one such explanation is that the alleles are a graded series of differentially severe mutations (or hypomorphs), such that a graded series of activities is created with the different developmental defects being assays of different sensitivity (CHOVNICK *et. al.* 1969). Accordingly, a mutation with a high amount of activity gives rise to only a few defects, while a mutation with

low activity gives rise to those same defects plus more defects. This can explain the expression of recessive visible phenotypes in heterozygotes containing a recessive visible and a lethal allele; however, it clearly does not explain complementation of eye/wing, eye–1/eye–2, and *l(1)N*/recessive visible heterozygotes. Another partial explanation considers the Notch gene as making a molecule with more than one functional site. Some mutations (most lethals) destroy the molecule completely or destroy a common site, while other mutations (recessive visible alleles or *l(1)N*) destroy a subset of the functional sites. However, the number of such independent sites required to account for all the independent developmental defects is large. Finally, it has been suggested (FOSTER 1973) that complementation be considered as product interactions in a multimeric protein: the heteromultimer for differently defective polypeptide subunits generates activity which neither homomultimer has (FINCHAM 1966). Although this might explain complementation, it does not explain how a single mutation can be defective in only a subset of the developmental events requiring the single Notch gene product.

ts mutations may identify a restricted coding region

Temperature-sensitive alleles are clustered (especially the ts lethals) in the right-most fifth of the locus, as are the majority of all mutations (Figure 1). Indeed, because fa^{no} exhibits highly variable expression at all temperatures, the only allele clearly expressing ts effects and mapping in the left four-fifths of the locus is the recessive visible *fa*. Clearly, the right-most fifth is more highly mutable, especially to ts alleles.

We believe that at least part of the ts mutable region, including specifically the $l(1)N^{ts1}$ site, encodes the functional product of this locus. This product is presumably a polypeptide. Firstly, EMS is thought to induce base substitutions (KRIEG 1963; SUZUKI 1970; SHELLENBARGER 1972) with temperature sensitivity due to inactivation of an altered polypeptide at high temperature (JOCKUSCH 1966). Although ts base substitutions of tRNA have been identified in *E. coli* (NAGATA and HORIUCHI 1973) and in yeast (RASSE-MESSENGUY and FINK 1973), Notch is genetically unrelated to such genes which are transcribed but not translated (presumptive tRNA's, RITOSSA, ATWOOD and SPIEGELMAN 1966; STEFFENSEN and WIMBER 1971; 5sRNA, WIMBER and STEFFENSEN 1970; and rRNA's, RITOSSA and SPIEGELMAN 1965). There is no evidence that DNA or an mRNA can be made temperature-sensitive by a single base substitution.

Secondly, conditional and therefore reversible expression of all the pleiotropy of the $l(1)N^{ts1}$ site mutation suggests that $l(1)N^{ts1}$ is not a nonsense or frameshift mutation because these would be irreversible. Therefore, $l(1)N^{ts1}$ is not acting through *cis* effects on function of other regions of the locus.

Thirdly, expression of mutations in the right-most fifth of the locus is generally conditional. Alleles exhibiting partial complementation, "contra-complementation", and temperature sensitivity for all effects map in this region. In addition, all recessive visible alleles in this region are conditional, expressing increased defects with temperature pressure. On the other hand, mutations out-

side this region are generally non-conditional, including *fa*^{9}, *spl*, *l(1)N*, $N^{264\text{-}39}$, $N^{264\text{-}40}$, and N^{55e11}. *fa* which shows a small change with temperature is unlike other ts recessive visible alleles in that no dramatic new phenotypes are observed at high temperature in homozygotes or in heterozygotes with N^{8} (Table 4). *fa*^{no} shows variable expression at all temperatures, and no new phenotypes are observed at high temperature in homozygotes.

Two models for genetic organization and control of Notch function in development

Two models for organization and control of a single functional product required in several independent aspects of development are suggested. These models state that mutants express selected defects due to critical developmental differences in the chemical conditions controlling synthesis (model I) or function (model II) of the Notch product.

Model I: control exerted at the level of transcription or translation: This model (after BRITTEN and DAVIDSON 1969) presumes there are several control regions which independently regulate production of the single functional gene product during development. Mutations of a specific control region act by blocking production of product only under the specific chemical conditions effecting regulation by that control region. In this model, *l(1)N*, *fa*^{9}, and *spl* would correspond to three independent control regions.

Model II: control exerted at the level of product function: This model presumes that each different mutation causes a different alteration of the functional product itself. Each altered product functions or does not function depending on the specific chemical conditions in each of the aspects of development in which the Notch product must function.

There are two critical differences between these models: (1) in model I, a mutant blocks production of functional product in *some* step in development, whereas in model II, a mutant produces an abnormal product in *each* step in development; and (2) in model I, mutations with selected defects should map outside the region that encodes the functional product, whereas in model II, these mutations should map within the region that encodes the functional product.

Because the product of the Notch locus has not been identified and can not be assayed directly by biochemical methods, definitive testing of models I and II is not possible at present.

It is interesting that the Notch locus appears to be too large to code for a single polypeptide by three criteria. Firstly, Notch mutants span a recombination distance of 0.14 map units (WELSHONS 1965). Assuming crossover frequencies are strictly proportional to nucleotide pairs, Notch is 15 times as long as the rosy gene (CHOVNICK *et al.* 1964). Secondly, Notch is the only gene in salivary chromosome band 3C7, an average size band containing presumably 3×10^{4} nucleotide pairs (RUDKIN 1965). Thirdly, Notch deficiencies approaching 3C7 from both sides can extend into the gene yet leave most of the 3C7 band and 3C7-8 interband (WELSHONS 1974). This indicates that the gene spans the band!

It is of critical importance to determine how the different mutations affect function in development, and thus distinguish between the two models presented above.

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